UNIVERSITY OF MUMBAI

Design and Development of a Novel Drug Delivery system for the treatment of Breast cancer

Submission of proposal for the registration of doctoral degree in the department of Science and Technology, University of Mumbai

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Title: Design and Development of a Novel Drug Delivery system for the treatment of Breast cancer

Aim of the study:

Design and development of a Novel drug delivery system that can be specifically targeted to the cancerous tumor without affecting healthy tissue.

To achieve better targeting ability for cancer prevention, and suppression of adverse side effects.

Objective of the study:

The objectives of the research work were as follows:

1. Selection, and procurement of extract, protein, and excipients.
2. Authentication of extract
3. Analytical method development
4. Preformulation screening in terms of solubility studies to find the solubility of the selected phytoconstituent in particular solvents, and drug-excipients compatibility studies by FTIR Spectroscopy.
5. Formulation Development
6. Optimization of formulation
7. Evaluation of Novel dosage form.
8. Stability studies as per ICH guidelines Long term 25℃+2℃/ 60%+ 50%RH, intermediate 30℃+2℃/60%+5%RH, and 40 C+2℃/75%+5%RH.
9. In-vivo studies: For conducting in-vivo studies permission will be obtained from Institutional Animal Ethics Committee (IEAC).
10. Evaluation of cytotoxicity studies.

Study Background (Literature Review) and significance of the study:

Cancer is one of the most prevalent causes of death across the globe. In 2020, around 2.3 million women were diagnosed with breast cancer which caused 685,000 deaths globally. By the end of 2020, there were 7.8 million women diagnosed and survived breast cancer in the past 5 years, making it the world's most extensive cancer. Breast cancer occurs in every country of the world

in women at any age after puberty but with increasing instances in later life. In India, one woman dies every 8 mins while diagnosed every 4 mins with breast cancer. Clinicians use a few core therapies alone or in combination with other therapy for higher-competence breast cancer treatment. Out of eight Indians, one is likely to develop cancer in their lifetime. In our country, still, a large number of patients consult doctors during the late phase of the disease. A few years ago, cervical cancer was prevalent among Indian women. But in the last few years, breast cancer cases are on the top of the table [1].

The proposed project focuses on formulating a novel dosage form for breast cancer. Herbs have the potential to provide the desired effect with minimum side effects. A combination of herbs with protein can enhance the efficacy of the formulation. We expect that the combination of herb with protein will enhance the therapeutic efficacy of the former.

National Status:

Pramod Kumar et. al., developed a mesoporous silica-based multifunctional nanocarrier for a combined delivery approach for drug-resistant TNBC cells. The loading of doxorubicin was modified in the formed nanocarrier to prevent interference with siRNA binding. The target specificity was analyzed by receptor blockade assay in cellular uptake studies. The nanocarrier showed a 40-fold reduction in IC 50 values when doxorubicin is coadministered with BCL-2 siRNA. The system was further validated using a 3-D in vitro cell culture [2].

Thakkar B et. al., developed liposomal gels by using liposomes entrapped with a herbal compound like seasamine, aloe-emodin, gallic acid, catechin, butein, 6-gingerol and curcumin as active drugs. Liposomal gels were prepared using carbopol with varying concentrations of propylene glycol, in other formulation mixture of HPMC, microcrystalline cellulose and sodium alginate were used proportionately. From the characterization of formulations, formulations with 1.5 % w/w carbopol and 5 % w/w propylene glycol have shown a significant release of bioactive. Besides this, the penetration effect of propylene glycol was also checked for topical liposome gel preparation; the data unveils that propylene glycol has no significant penetration-enhancing effect with liposomal gel preparation [3].

Verma et al., showed that the peritoneal metastasis of TNBC, particularly splenic, is facilitated by the preferential hyperactivation of operational EZH2 (H3K27me3) over NC-EZH2. By weakening the binding of the regulator SP1 to the promoter of KRT14, it promotes transcription instead of H3K27me3-mediated suppression of gene expression. Furthermore, migration, invasion, and peritoneal metastasis of TNBC are all markedly diminished by KRT14 deletion. Human TNBC metastasis frequently exhibits a favorable connection between KRT14 and H3K27me3 levels [4].

Mehraj et al., identified the molecular pathways that decrease tumorigenicity as well as the cytotoxic activities of adapalene in TNBC cells and its efficacy when combined with doxorubicin. Adapalene suppressed the development and proliferation of tumor cells and collaborated with doxorubicin to prevent the expansion, colony formation, and migration of TNBC cells. Additionally, the two drugs together increased the production of reactive oxygen species, which led to the hyperphosphorylation of Erk1/2 and caspase-dependent death. The findings also show that adapalene is a potent anticancer drug that can be employed alone or in combination with currently available therapy regimens to treat TNBC [5].

International Status:

Akbarzadeh et. al provided an effective perspective on breast cancer therapy as a malignant disease. The curcumin-Loaded Niosome calcium Alginate Nanocarrier approach does not have any adverse effects or drug resistance on cancer patients. This system requires utilizing nano drugs that are laden into a nano-carrier. In this study, niosome calcium alginate as a nanocarrier that consists of curcumin was formulated with the aim of eliminating breast cancer. The formulation was applied to breast cancer cell lines that were SKBR3 and MDA-MB231 and evaluated the gene expression levels to assess the efficacy of this novel approach. Interestingly, the application of this formulation increases cancer cell death and apoptosis. This approach could be utilized as a successful and novel aspect of breast cancer treatment [6].

Curcio et al., researched CD44-receptors and double stimuli (pH/redox)-responsive nanoparticles as a delivery option for doxorubicin hydrochloride in receptor-less breast cancer in-vitro studies. A phosphatidylcholine lipid layer was moistened with a solution of oxidized hyaluronic acid and doxorubicin, selected as the model drug, and then a crosslinking reaction

with cystamine hydrochloride was carried out. The produced spherical nanoparticles were found to be successfully incorporated in cancer cells by receptor-mediated endocytosis and to regulate the drug release reliant on the pH and redox potential of the surrounding medium. In-vitro cytotoxicity assays revealed the safety and efficacy of the nanoparticles in elevating the cytotoxic effect of the unbound anticancer drug, with the IC50 values being decreased in MDA-MB-468 and MDA-MB-231. The fusion of self-assembled phospholipid molecules with a polysaccharide equivalent acting as a receptor ligand, and stimuli-responsive chemical component, was performed on multifunctional nanoparticles able to actively target breast cancer cells and enhance the in-vitro anticancer activity of doxorubicin [7].

Vivo-Llorca et al., reported here the creation and assessment of an innovative nanodevice enzyme function-dependent gold nanoparticles to effectively carry out enzyme prodrug treatment (EPT) in breast tumor cells as a way to overcome the limits of conventional therapy. Horseradish peroxidase (HRP) is an enzyme that can be added to gold nanoconjugates to provide effective nanoplatforms for EPT. Breast cancer cells efficiently absorb the nanodevice since it is biocompatible. Surprisingly, co-treatment with IAA and HRP-AuNCs (HRP-AuNCs/IAA) lowers the proliferation of breast carcinoma cells to less than 5%. A 74% reduction in cell viability is observed in 3D tumor models (multicellular tumor spheroid-like cultures) co-treated with HRP-AuNCs/IAA, but not in free-formulated components (HRP, IAA). Overall, the findings suggest that the developed HRP-AuNCs nanoformulation has exceptional therapeutic efficacy. These results could pave the way for developing nanosystems for EPT in breast cancer, which would help to overcome the development of Nanosystems for EPT in breast cancer, which would help to get over the clinical constraints of existing tumor enzyme therapy [8].

Gi Ru Shin et al., researched Resveratrol (Res), which possesses chemotherapeutic and metastasis inhibitory actions, and may be used intravenously to treat TNBC. In order to create a Res-Cx-HA reservoir inside the tumors, a parenteral Res-loaded crosslinked hyaluronic acid hydrogel (Res-Cx-HA) was created. The Res-Cx-HA formulation had good tumor tissue injectability, and fast depot development within the tumor, and the depot stayed inside the injected tumor for a long time. Res-Cx-HA depots that were created in vivo kept Res inside the tumor for a long time. Res' bioavailability and therapeutic effectiveness remained largely confined to the tumor and did not spread to other organs. Injections of Res-Cx-HA into tumors in

animal models caused significant reductions in tumor growth rates, as well as a significant number of apoptotic cells and minimal angiogenesis [9].

Fu Hou Wong et al., studied the combined effect of calcitriol and talazoparib cell migration in, apoptosis, proliferation, and cell cycle in cell lines. In MDA-MB-468 and BT-20 cell lines, monotherapies and their combinations were examined for their effects on (i) cell proliferation (using a real-time cell analyzer assay), (ii) cell migration (using a CIM-Plate assay), and (iii) apoptosis and cell cycle assessment (using flow cytometry). Talazoparib and calcitriol were at their most effective antiproliferative concentrations in BT-20 at 91.6 and 10 M, respectively, and in MDA-MB-468 at 1 mM and 10 M. Combination therapy dramatically enhanced the ability of both cell lines to suppress cell migration. The combination treatment in BT-20 dramatically raised S and G2/M groups, as well as late apoptosis. In comparison to the control, combined therapy in MDA-MB-468 considerably boosted the S population and lowered G0/G1. Combination therapy significantly elevated necrosis, and early, and late apoptosis in MDA-MB-468. Combining talazoparib and calcitriol profoundly impacts profound impact on TNBC cell line proliferation, migration, induction of apoptosis, and necrosis. This mixture may be effective in the treatment of TNBC [10].

Study Hypothesis: The proposed study will be optimized using the Quality by design (QbD) approach. Quality by design is a scientific perspective by which formulations are designed to gain deliberate product quality. As per the QbD theory, desirable quality can be achieved if crucial factors applicable to the output variability are recognized and suitably removed or maintained inside a pre-established design area. This perspective comprises the arithmetic design of experiments (DoE) to systematically optimize nanocarriers. The trials will be designed using Design of Experiments (DoE) using Design Expert Software. In this design, 2^3 factorial methods with 4 centre points will be studied. The factors will include critical process parameters of formulation whereas the response to be analyzed shall be corresponding evaluation parameters.

Study Rationale: Triple-Negative Breast Cancer (TNBC) refers to the cancer cells that don't have estrogen or progesterone receptors (ER or PR) and also don't produce HER2 protein. TNBC is the main form that occurs in women at high genetic risk counting those with mutated BRCA1 genes and also women in the post-menopausal phase. There is a steep increase in cases of Breast

cancer globally. TNBC accounts for about 10-15% of total breast cancer cases. Researchers are engrossed in breast cancer treatment and mitigation including minimizing the adverse effects of conventional treatment on a large scale.

The selected herb is a promising herb, consisting of phytoconstituent which shows positive results. Clinical evaluation of herbs is ongoing for the treatment of a range of other skin cancers in humans, and related animals, and is showing an optimistic safety and efficacy profile [11]. Although a lot of ongoing research in the field of cancer with herbs, it is yet unexplored in the region of Breast cancer.

Also, the selected protein is an excellent carrier for bioactives. It also migrates to tumor cell nuclei to cause disruption of chromatin, loss of transcription, and nuclear condensation. It is used in various combinations like gold and silver nanoparticles, conjugations, and micelles formations [12].

Despite tremendous investigation on the selected herb and protein, a combination of both is still unexplored. Hence the proposed project aims towards preparing a stable, patient-compliant, nano-formulation for the treatment of Breast cancer by combining them. The research targets patients suffering from various types of Breast cancer, including, the most aggressive triple-negative breast cancer and metastatic Breast cancer. It also targets Ductal carcinoma, which is the most common Breast cancer.

Research Methodology:

Material Drug and excipients: Suitable herbs and excipients will be procured/obtained from Pharma-grade-suitable manufacturers. All the other excipients and reagents will be of analytical grade.

Method

1. Literature survey: Extensive Literature search will be carried out to find out the suitable herb, excipients, and chemicals and also to find out that no work on the proposed research was carried out elsewhere.
2. Selection, and procurement of extract, protein, and excipients.
3. Authentication of extract
4. Analytical method development

5. Preformulation screening in terms of solubility studies to find out the solubility of the selected phytoconstituent in particular solvents, and drug-excipients compatibility studies by FTIR Spectroscopy.
6. Formulation Development
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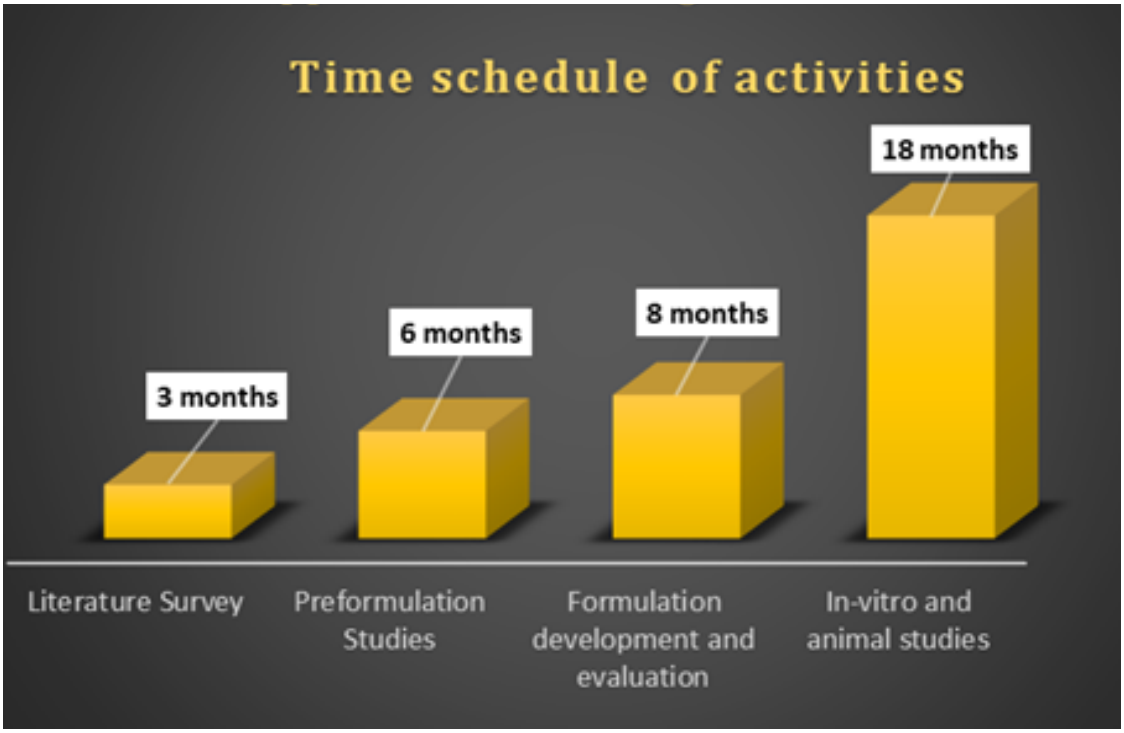
9. Stability studies as per ICH guidelines Long term 25℃+2℃/ 60%+ 50%RH, intermediate 30℃+2℃/ 60%+ 5%RH, and 40 C+ 2℃/ 75%+ 5%RH.
10. In-vivo studies: For conducting in-vivo studies permission will be obtained from Institutional Animal Ethics Committee (IEAC).
11. Evaluation of cytotoxicity studies.
12. Data compilation & Thesis writing

Timeline of the project:



Statistical plan: ANOVA will be used to interpret the statistical results. The prospective studies that conduct an interim analysis according to re-determined criteria while the study is ongoing are beneficial for scientific research. More important than p values are the confidence intervals

surrounding the parameter estimates being reported. The confidence interval is designated prior to examining the data and is conventionally set to 95%. When false positives (type I errors) are a greater concern, the confidence interval of 99% will be considered.

Limitations of the study: The limitation of the proposed work is clinical trials. Clinical trials are not possible due to lack of time and the funding required for them is not available.

Expected outcomes of the study:

1. The proposed investigation of a novel formulation of the herb for breast cancer will provide the therapeutic efficacy of such a system.
2. Helps in treating patients suffering from breast cancer in women from both rural and urban areas.
3. Cost-effective and easy availability of Novel formulation.
4. Formulation consideration will be considered by trying different combinations of excipients, equipment, and processing parameter.
5. Characterization of formulation in terms of size, and cell line studies.
6. Stability studies according to ICH guidelines will be conducted to confirm the storage condition of the optimized formulation.

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